--The second oliog. was in the reverse orientation and spanned a unique Pmel site (underlined) (SEQ ID NO: 27):

5'-gcccagtgtttaaacactcgag-3'--

REMARKS

Formal examination of this application is respectfully requested.

Figures 2-4 and 6-8 and the specification were amended to recite sequence ID numbers for the listed sequences.

As the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Respectfully submitted,

Date November 29, 2001   By _Michele M Simkin_

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"Version of the Specification with Markings to Show Changes Made"

Page 63, please replace the eighth full paragraph with the following:

--Figure 5 which shows the sequence of MLV pICUT **(SEQ ID NO: 1)**;--

Page 64, please replace the third full paragraph with the following:

--Figure 11 which shows the Peicut-*LacZ* sequence **(SEQ ID NO: 2)**;--

Page 64, please replace the seventh full paragraph with the following:

--Figure 15 which shows a sequence comparison of mutant *env* (m4070A) **(SEQ ID NO: 5)** with wild type MMLV sequence from the 3' end of the *pol* gene **(SEQ ID NO: 4)**;--

Page 64, please replace the eighth full paragraph with the following:

--Figure 16 which shows the complete sequence **(SEQ ID NO: 3)** of the modified *env* gene m4070A;--

Page 65, please replace the fourteenth full paragraph with the following:

--Figure 33 which shows the pTRONIN sequence **(SEQ ID NO: 21)**;--

Page 66, please replace the third full paragraph with the following:

--Figure 36 which shows the pTRONIN-1 sequence **(SEQ ID NO: 22)**;--

Pages 68-69, please replace the paragraph bridging the two pages with the following:

--Figure 14 shows a schematic diagram of the construction of a MLV pICUT *Neo*-p450 vector that restricts hygromycin expression to producer cells and 2B6 (a p450 isoform) expression to transduced cells. The starting vector for this construction is the pICUT vector of Figure 13 containing both *hygro* and *neo*. The *neo* gene is replaced with the complete p450 2B6 cDNA as follows: The complete 2B6 cDNA is obtained by RT-PCT on human liver RNA (Clontech) using the following primers **(SEQ ID NOS 41 and 42, respectively, in order of appearance)**:--

Pages 71-72, please replace the paragraph bridging the two pages with the following:

--PPTEIAV+ (Y8198) (SEQ ID NO: 18): GACTACGACTAGTGTATGTTTAGA AAAACAAGG,

and

3'NEGSpeI(Y899) (SEQ ID NO: 19): CTAGGCTACTAGTACTGTAGGATCTCGAACAG--

Page 72, please replace the fourth full paragraph with the following:

--VSAT1 (SEQ ID NO: 20): (GGGCTATATGAGATCTTGAATAATAAAATGTGT) and VSAT2 (SEQ ID NO: 6): (TATTAATAACTAGT) and

pHIT60 (Soneoka et al. 1995 Nucleic Acids Res 23: 628-633) as template. The product is digested with BglII and SpeI and cloned into the BglII/SpeI sites of pCIE-Neo.--

Page 72, please replace the sixth full paragraph with the following:

--CMV5'EIAV2;

(Z0591) (SEQ ID NO: 7) (GCTACGCAGAGCTCGTTTAGTGAACCGGGCACTCAGATTCTG (sequences underlined anneals to the EIAV R region)

and

3'PSI.NEG (SEQ ID NO: 8) (GCTGAGCTCTAGAGTCCTTTTCTTTTACAAAGTTGG).—

Page 77, please replace the fourth full paragraph with the following:

--A DNA fragment containing the LTR and minimal functional packaging signal is obtained from the retroviral vector MFG (Bandara et al 1993 Proc Natl Acad Sci

90:10764-10768) or MMLV proviral DNA by PCR reaction using the following oligonucleotide primers:

_Hind_IIIR **(SEQ ID NO: 9)**: GCATTAAAGCTTTGCTCT

L523 **(SEQ ID NO: 10)**: GCCTCGAGCAAAAATTCAGACGGA

Page 78, please replace the sixth full paragraph with the following:
--1) The starting plasmid for the construction of this vector is pLNSX (Miller and Rosman 1989 BioTechniques 7: 980-990). The natural splice donor (...agGTaag...) contained within the packaging signal of pLNSX (position 781/782) is mutated by PCT mutagenesis using the ALTERED SITES II mutagenesis kit (Promega) an a synthetic oliognucleotide of the sequence **(SEQ ID NO: 11)**:
5'-caaccaccgggagGCaagctggccagcaactta-3'—

Page 79, please replace the first full paragraph with the following:
--2) A CMV promoter from the pCI expression vector (Promega) is isolated by PCR using the following two oligonucleotides:

Primer 1 **(SEQ ID NO: 12)**: 5'-atcggctagcagatcttcaatattggccattagccatat-3'

Primer 2 **(SEQ ID NO: 13)**: 5'-atcgagatctgcggccgcttacctgcccagtgcctcacgaccaa-3'—

Page 79, please replace the third full paragraph with the following:
--3) The 5' end of a cytochrome P450 cDNA coding sequence is isolated by RT-PCR from human liver RNA (Clontech) with the following primers:

Primer 3 **(SEQ ID NO: 14)**: 5'-atcggcggccgcccaccatggaactcagcgtcctcct cttccttgcaccctagg-3'

Primer 4 **(SEQ ID NO: 15)**: 5'-atcggcggccgcacttacCtgtgtgccccaggaa agtatttcaagaagccag-3'—

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Page 80, please replace the first full paragraph with the following:

--5) The 3' of the P450 coding sequence is isolated by RT-PCR amplification from human liver RNA (Clontech) using the following primers:

Primer 5 **(SEQ ID NO: 16)**: actgtgatcataggcacctattggtcttactgacatccacttt ctctccacagGcaagtttacaaaacctgcaggaaatcaatgcttacatt-3'

Primer 6 **(SEQ ID NO: 17)**: actgatcgatttccctcagccccttcagcggggcaggaagc-3'—

Page 84, please replace the first full paragraph with the following:

--Finally for the PCR reaction, two primers were used. The first (primer A1) was designed to anneal downstream of the U3 start of transcription but upstream of the small T SD sequence **(SEQ ID NO: 23)** (5'-gttaacactagtaagcttg-3'). The second primer (primer A2) was designed to anneal downstream of the splice acceptor in the reverse orientation **(SEQ ID NO: 24)** (5'-gattaagttgggtaacgccaggg-3'). These primers would therefore amplify between the region from upstream of the small T splice donor to downstream of the splice acceptor. Consequently this PCR reaction would pick up both full-length and spliced message (see Figure 34).—

Page 84, please replace the second full paragraph with the following:

--Once complete, the PCR reaction was separated on an 1% agarose gel. This analysis revealed there to be two products from the pTRONIN transduced cells. Both of which were smaller than full length transcripts, suggesting splicing had occurred. Both fragments were gel extracted, cloned and sequenced to reveal that one product was a transcript generated by a **splicing** [spliceing] reaction between the small T splice donor (copied to the 5' LTR during reverse transcription) and splice acceptor. The other, larger product instead contained a **splicing** [spliceing] event between the splice acceptor and a previously unidentified cryptic splice donor contained within the packaging signal (mapping to position 810-811 of wild type MLV; the sequence context being **(SEQ ID NO: 25)** cagGTtaag (with the GT splice donor in bold).--

Page 84, please replace the third full paragraph with the following:

--To investigate this cryptic splice donor futher, it was mutated in pTRONIN by the following method: First two olgos. were synthesized. The first spanned both the unique BstEII site and the GT of the cryptic splice donor. This oligo. contained a splice donor point change (shown in bold) of GT to GC. The BstEII site is shown as uppercase. Its sequence is shown below **(SEQ ID NO: 26)**:--

Page 85, please replace the second full paragraph with the following:

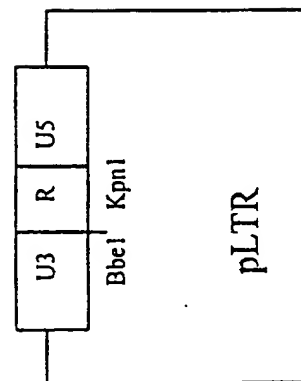--The second oliog. was in the reverse orientation and spanned a unique PmeI site (underlined) **(SEQ ID NO: 27)**:

5'-gcccagt<u>gtttaaac</u>actcgag-3'--

Figure 2

Bbe1
overhang

Splice donor
(underlined)

Start of MLV R
(in italics)

Kpn1
overhang

(SEQ ID NO: 28)CGTTAACACTAGTAAGCTTGCTCTAAGGTAAATAGTCGACAGGCCTGCGCCCAGTCCTCCGATTGACTGAGTCGCCCGGGTAC
CGCGGGCAATTGTGATCATTCGAACGAGATTCCATTTATCAGCTGTCCGGACGCGGTCAGGAGGCTAACTGACTCAGCGGGCCC (SEQ ID NO: 29)

U3 | R | U5

Bbe1 | Kpn1
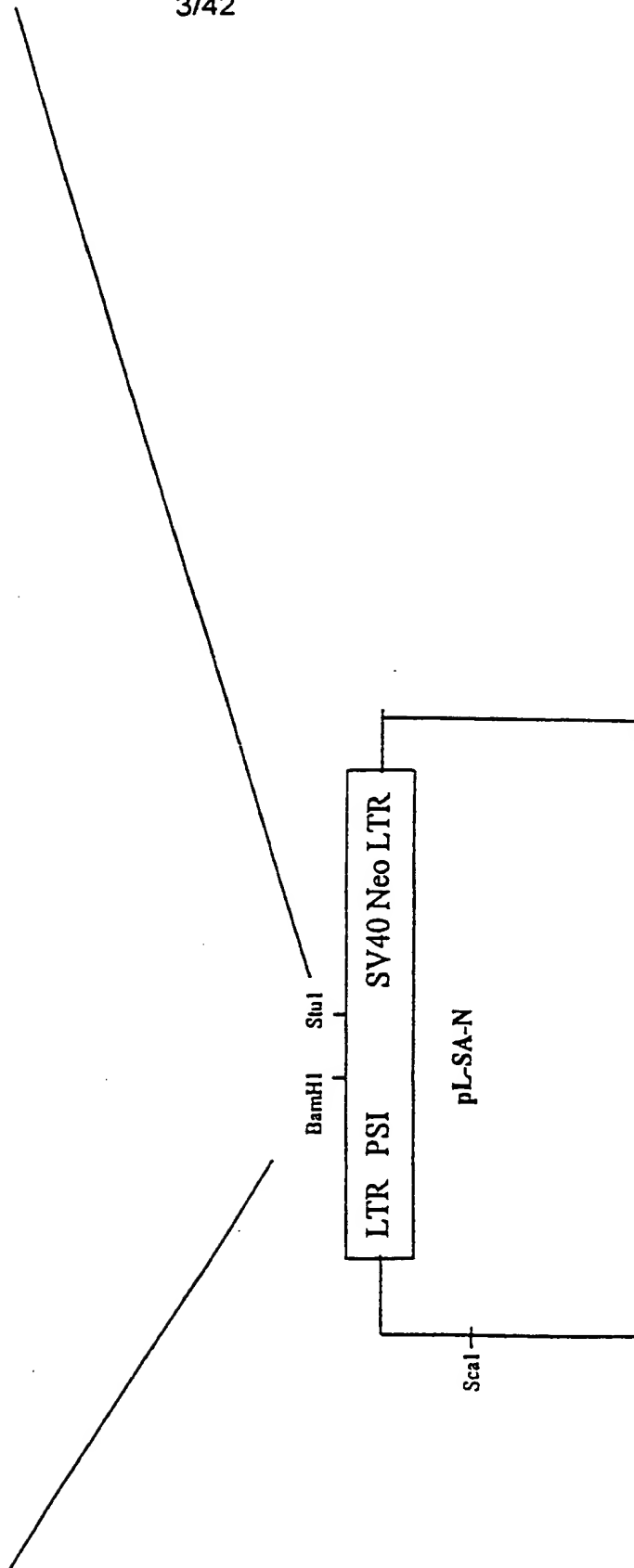
pLTR

Figure 3

SA ▼

Branch point ▶

(SEQ ID NO: 30)
5'-GATCTAACCTAGGTCTTCGAGTGTTGTTTAAACACTGGGCTTGTCTCGAGACAGAGAAGACTTCTTGCCGTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGAGG
ATTGGATCCAGAGCTCACAAATTTGTGACCCGACCTATCCGTGGATAACCAGAATGACTGTAGGTGAAACGGAAAGAGAGGTGTCCACTCC

BamHl Stu1
LTR PSI   SV40 Neo LTR
               pL-SA-N
Scal

Figure 4

Spe1

(SEQ ID NO: 31)

GT TO GC CHANGE

3'-GGTGGCCCTCCGTTCGACCGGTCGTTGAATAGACACAGACAGGCTAACGATACACAGATACAAACTACAATACGGCGGACGCAGACATGATCAATCGATT-5'

Asc1

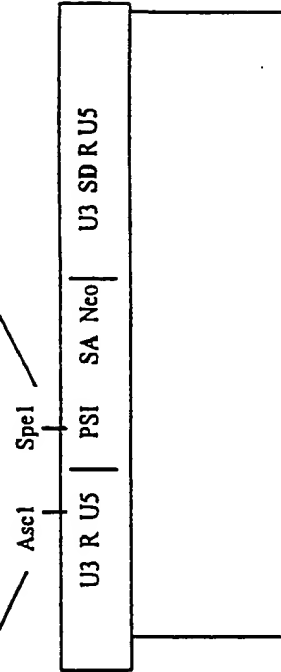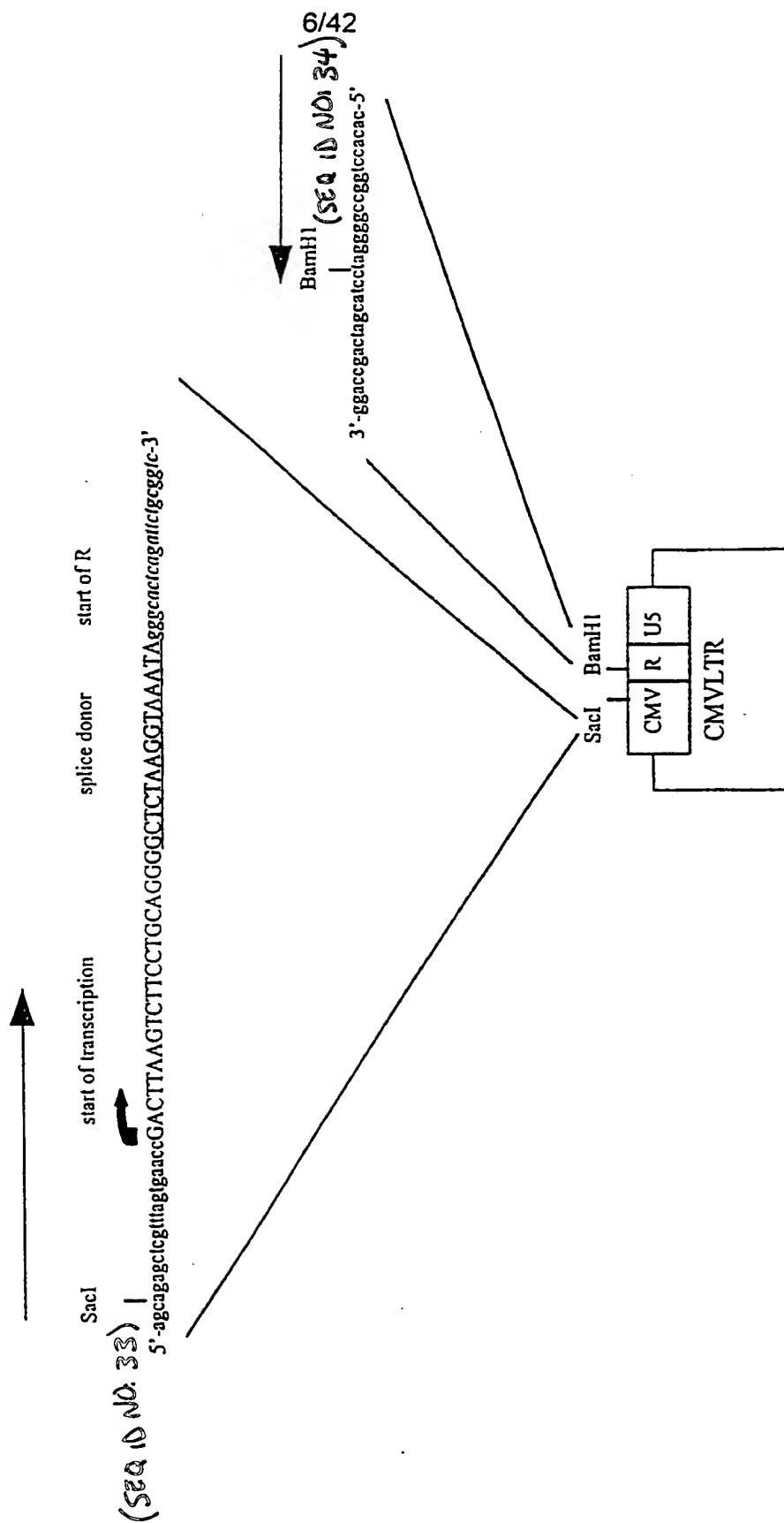(SEQ ID NO: 32)

5'-CCCTCACTCGGGCGCGCCAGTCTTCCGA-3'

| U3 R U5 | PSI | SA Neo | U3 SD R U5 |

Asc1 Spe1

6/42

Figure 6

(SEQ ID NO. 33)

SacI

start of transcription   splice donor   start of R

5'-agcagagctcgtttagtgaaccGACTTAAGTCTTCCTGCAGGGGCTCTAAGGTAAATAgggcactcagattctgcggtc-3'

BamHI (SEQ ID NO. 34)

3'-ggaccgactagcatcctaggggccggtccacac-5'

SacI   BamHI

| CMV | R | U5 |

CMVLTR

Figure 7

Xhol
overhang

(SEQ ID NO: 35)

5'-TCGACGTTTAAACACTGGGCTTGTCTGAGACAGAAGACTCTGCGTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCCACAGGTCACGTGAAGCTAGCCTCGAGTTGGC-3'
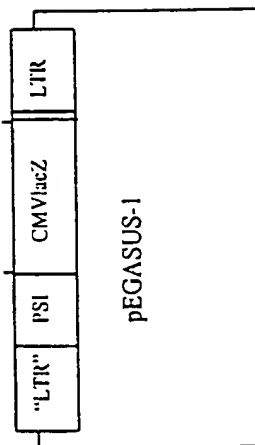
3'-   GCAAATTTGTGACCCGAACAGCTCTGTCTTCTGAGAAGCAGAAATGACTGTAGGTGAAACGGAAAGAGAGGTGTCCAGTGCACTTCGATCGGAGCTCAACCGACT

(SEQ ID NO: 36)

Branch point

SA ▶

Bpul102 overhang

Xhol   Bpul102

"LTR" PSI CMVlacZ LTR

pEGASUS-1

Figure 8

Oligo1 (SEQ ID NO: 37)

5'-aggaggacaggCaagatgggagaccctttgac-3'

Oligo2 (SEQ ID NO: 38)

3'-ctttcctgagatctcagctgggg-5'
Sal1

Oligo3 (SEQ ID NO: 39)

3'-tcctcctgtccGtctaccctctgggaaactg-5'

Oligo4 (SEQ ID NO: 40)

5'-ctatataagcagagagctcgtttagtg-3'
SacI

SD

SacI|Sal1

"LTR"	PSI	IgSA	LTR

pEGASUS+SA